# COMPARISON OF FOUR PLATING TECHNIQUES AND FOUR MEDIA FOR THE ENUMERATION OF HETEROTROPHIC BACTERIA



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# COMPARISON OF FOUR PLATING TECHNIQUES AND FOUR MEDIA FOR THE ENUMERATION OF HETEROTROPHIC BACTERIA

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# ABSTRACT

Three experiments were conducted to provide information for the selection of a method for the isolation and enumeration of heterotrophic bacteria in surface waters. The experiments involved studies of three factors:

1) Plating Technique (pour plate, spread plate, spot plate and membrane filtration).
2) Addition of 100 ppm Actidione to control mold contamination.
3) Media Formulation (Modifications of the Foot and Taylor Agar and CPS Agar).

Samples were obtained from two of the Great Lakes and four rivers. All experiments were set up to allow a thorough statistical evaluation of the data.

The results of the statistical analysis of the data demonstrated that:

1) The spot plate and spread plate techniques gave similar recoveries which were significantly higher than those obtained by use of the membrane filtration and pour plate techniques.
2) Actidione could be added to the media at a concentration of 100 ppm without reducing recoveries.
3) The geometric mean recovery was highest on the CPS Agar.

The only media, however, that had a significantly lower geometric mean recovery was the Foot and Taylor Agar with the lowest peptone concentration (0.5% (w/v)). Non-parametric analysis of the data demonstrated that the CPS agar had the highest heterotrophic bacterial recoveries significantly more often than any of the other media.

Therefore, as a general purpose method for the isolation and enumeration of heterotrophic bacteria, a combination of the spot plate technique in conjunction with CPS Agar + 100 ppm Actidione is suggested. This is not, however, recommended for all situations as it was apparent that under certain circumstances other techniques, such as the spread plate and/or a different media may be more suitable.

# COMPARISON OF FOUR PLATING TECHNIQUES AND FOUR MEDIA FOR THE ENUMERATION OF HETEROTROPHIC BACTERIA.

M. Young

## Introduction

The Great Lakes Microbiology Laboratory began analyzing a large number of samples for heterotrophic bacteria in 1973. Previous to that time there was no routine method in use within the Ministry of the Environment, therefore, a method was developed based on earlier Great Lakes work. The plating method adopted was membrane filtration onto black membranes (Bennett, 1969). The medium chosen for incubation of the filters was a modified Foot and Taylor agar (Foot and Taylor, 1949; Bell and Dutka, 1972) containing 0.5% (w/v) casein. Results obtained during 1973 indicated that the black membranes were inhibitory. In many instances it was found that the non-coliform counts on the total coliform media (m-Endo LES (Difco)) were equivalent to or greater than the heterotroph counts (Clark, 1973 - Personal Communication). The inhibitory nature of the filters was confirmed with data from recreational lakes (Hendry, 1976). The problem of inhibition made it necessary to investigate a new procedure. A small preliminary experiment at this laboratory (Unpublished) demonstrated that the use of a spot plate technique (S) (Gaudy, et al., 1963; Bousfield, et al., 1973) produced higher counts than the counts by membrane filtration (MF) on both Foot and Taylor agar (S/MF = 1.95) and Nutrient agar (S/MF = 1.26) and that, when the spot plate technique was employed, counts on Foot and Taylor (FT) agar exceeded those on Nutrient agar (N) (FT/N = 1.51). Therefore, a combination of the spot plate technique and Foot and Taylor agar were chosen for further study. Problems were almost immediately encountered with spreading colonies overgrowing the spots. To combat this problem, the agar concentration was increased from 1.5% to 2.0% (w/v) and the casein concentration was decreased from .5% to .05% (w/v). The problem of spreading colonies was controlled by these modifications, however, a

further problem of contamination with mold was noted. In addition, some published literature (Collins and Willoughby, 1962; Jones, 1970 and Staples and Fry, 1973) suggested that a variation of the medium containing starch and glycerol (CPS agar) provided higher counts in surface waters of lakes and rivers.

Because of these findings, the present study was initiated for the following reasons:

(1) to compare, more thoroughly, the spot plate technique with other plating methods to determine whether it was efficient and practical,

(2) to compare modifications of the basic Foot and Taylor medium to determine which formulation provided the highest recoveries of heterotrophic bacteria from environmental water samples and

(3) to determine if Actidione could be incorporated into the medium to prevent mold contamination.

## Methods

Three separate studies were carried out as follows:

1) Comparison of Plating Techniques

(a) A total of 84 surface water samples submitted to the Great Lakes laboratory for routine analyses were used for this experiment. The sampling areas and number of samples from each location were as follows:

...3

| | | |
|---|---|---|
| Lake Ontario Waterfront Samples | | 53 |
| Burlington | 8 | |
| Etobicoke | 9 | |
| Toronto | 30 | |
| Scarborough | 6 | |
| Hamilton Harbour | | 15 |
| Humber River (mouth) | | 5 |
| Humberview Park | | 5 |
| Don River (Danforth Ave.) | | 6 |
| | | 84 |

Heterotrophic bacterial densities (HB) were determined using a modified Foot and Taylor agar containing 0.05% peptone and 100 ppm Actidione. Counts were obtained after 7 days of incubation at 20°C.

The following four techniques were compared:

i) Pour Plate:

1.0 ml of a sample and/or sample dilution was aseptically pipetted into a previously sterilized round glass petri dish. Approximately 20 ml of the molten agar medium, cooled to 45°C, was then poured into the petri dish and the contents were mixed thoroughly with a gentle swirling motion.

ii) Spread Plate:

0.1 ml of a sample and/or sample dilution was pipetted onto a pre-dried agar plate and spread evenly over the surface with sterile bent glass rod. The petri dish contained approximately 20 ml of medium which was pre-dried at room temperature by placing it, with the cover removed, for one hour in a laminar flow unit.

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iii) Membrane Filtration:

1.0 ml of the sample and/or sample dilution was filtered using white gridded 0.45 u membrane filters (Millipore HAWG). The membrane was placed on agar plates for incubation.

iv) Spot Plate:

Two separate 0.1 ml aliquots of the sample and/or sample dilutions were pipetted on pre-dried agar plates (see Spread Plate) and allowed to absorb into the medium without spreading the aliquot over the plate. The results were based on an arithmetic average of the two spots.

Analyses were set up based on a Latin Square organization (Table I), to compensate for the effect of time between the first and last analyses. The "square" was repeated for each set of four samples.

Table I: Order of Analysis for Plating Experiment

| Sample Number | Analytical Order<br>1 | 2 | 2 | 4 |
|---|---|---|---|---|
| 1 | Pour | Spread | MF | Spot |
| 2 | Spot | Pour | Spread | MF |
| 3 | MF | Spot | Pour | Spread |
| 4 | Spread | MF | Spot | Pour |
| . | | | | |
| . | | | | |
| . | | | | |

b) In order to determine if the counts in the two spots from the spot plate technique differed significantly, the $D^2$ Index of Dispersion Test was applied (see Section 4, Statistical Methods).

The counts from 393 determinations were analyzed using this method. The determinations tested were as follows:

| | |
|---|---|
| Preliminary Media Comparison | |
| Formulation Experiment | 96 |
| Lake Ontario | 200 |
| Lake Erie | 55 |
| Toronto Harbour | 42 |
| | 393 |

2. Preliminary Media Comparison Experiment

Three samples were used for this experiment, one each from the Rouge River, Humber River and Lake Ontario.

Eight media formulations were tested (Appendix, Page 28)

| | | |
|---|---|---|
| (a) Foot & Taylor | 0.5 g peptone | with 100 ppm Actidione |
| (b) Foot & Taylor | 0.5 g peptone | without Actidione |
| (c) Foot & Taylor | 1.0 g peptone | with 100 ppm Actidione |
| (d) Foot & Taylor | 1.0 g peptone | without Actidione |
| (e) Foot & Taylor | 3.0 g peptone | with 100 ppm Actidione |
| (f) Foot & Taylor | 3.0 g peptone | without Actidione |
| (g) CPS Agar | | with 100 ppm Actidione |
| (h) CPS Agar | | without Actidione |

All analyses were performed utilizing the spot plate technique with incubation at 20°C for seven days.

Each sample was analyzed in duplicate by four technicians on each of the eight media. The order in which each technician used the eight media was randomized, by use of random number generation on a Hewlett-Packard Model 9830 A calculator, to compensate for any possible effect of the time between the first and last analysis.

3. Second Media Comparison Experiment

A total of 109 routine surface water samples submitted to the Great Lakes laboratory were analyzed in this experiment. The sampling areas and number of samples from each location are as follows:

| | | |
|---|---|---|
| Lake Ontario Waterfront Samples | | 73 |
| Burlington | 17 | |
| Peel | 6 | |
| Etobicoke | 18 | |
| Toronto | 20 | |
| Scarborough | 12 | |
| Hamilton Harbour | | 8 |
| Lake Ontario | | 12 |
| Twelve Mile Creek | | 16 |
| | | 109 |

In this experiment, the media containing 100 ppm Actidione (see p. ) were compared. The agar concentration in the CPS media was increased to that of the other media (2.0% (w/v)).

All analyses were carried out employing the spot plate technique with incubation at 20°C for seven days.

Analyses were organized as in the plating experiment, based on the Latin Square (Table II):

Table II: Order of Analysis for Second Media Comparison

| Sample Number | Analytical Order 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 0.5 g | 1.0 g | 3.0 g | CPS |
| 2 | CPS | 0.5 g | 1.0 g | 3.0 g |
| 3 | 3.0 g | CPS | 0.5 g | 1.0 g |
| 4 | 1.0 g | 3.0 g | CPS | 0.5 g |
| . | | | | |
| . | | | | |
| . | | | | |

The analytical pattern was repeated for every four samples.

## 4. Statistical Methods

The log transformed data from all three experiments were first analyzed by a factorial analysis of variance (ANOVA) on a Hewlett-Packard Model 9830 A calculator. When this was completed, the geometric means (GM) obtained for the various techniques or media tested were compared using Tukey's t - test:

$$t = \frac{m_1 - m_2}{\sqrt{\frac{\left[ 2 \times MS\ (Error) \right]}{n}}}$$

for four treatments (MS (Error) was used as calculated by the ANOVA.)

The data from the second media formulation study was also analyzed for performance over different log GM ranges and over different bodies of water by the test for ordered alternatives based on the Friedman Rank Sums (FRS) (Hollander and Wolfe, 1973).

A comparison of the two spots counted for the spot plate technique was obtained using the Index of Dispersion (Eisenhart and Wilson, 1943):

$$D^2 = \frac{(x - \bar{x})^2}{\bar{x}}$$

## Results and Discussion

### 1. Plating Technique

(a) The ANOVA table generated from the factorial analysis is included in the Appendix, Table A. The results of the ANOVA indicated a significant difference at the 5% level between the samples utilized and the plating techniques.

The log GM recovery of the HB for each method used was:

Table III: Log GM Recoveries of HB by Four Plating Techniques

| Technique | Log GM |
|---|---|
| Pour | 4.3169 |
| Spread | 4.8397 |
| MF | 4.6228 |
| Spot | 4.9588 |

The results of the Tukey's t - test are included in the following table where n = 84, t (0.05) = 2.63 and MS (Error) = 0.1339:

Table IV: Results of Statistical Comparison of HB Recoveries

| Method | Pour | Spread | MF |
|---|---|---|---|
| Spread | 9.25*(SD) | | |
| MF | 5.41 (SD) | 3.84 (SD) | |
| Spot | 11.36 (SD) | 2.11 (NSD) | 5.95 (SD) |

SD = Significantly different

NSD = Not significantly different.

A previous study on the spread plate technique indicated that there would be a loss of viable bacteria as a result of carryover on the spreader (Hendry, 1966 - Personal Communication). The amount of carryover varied with the volume of liquid being spread and the size of the spreader. In the present study it was estimated that the loss would be approximately 10%. In order to determine if this would effect the experiment, the spread plate counts were adjusted upwards by 10% giving a GM of 4.8811. When the same statistical procedure was applied, it was found that the adjustment did not significantly change the results (Table V).

Table V: Comparison of Plating Techniques Using the Adjusted Spread Plate GM

| Method | Pour | MF | Spot |
|---|---|---|---|
| Adjusted Spread Plate | 9.99 (SD) | 4.58 (SD) | 1.38 (NSD) |

The range of recoveries in comparing the four techniques was spread over less than one log (4.32 - 4.96), however, sufficient data was analyzed to determine that the spot plate technique had comparable recoveries to the spread plate technique and that both had higher recoveries than the MF technique. Lowest recoveries were obtained with the pour plate technique.

The spot plate technique has certain advantages over the spread plate technique in that it can be performed more rapidly and required less media. There are, however, certain instances indicated by the Index of Dispersion when some other approach may be necessary.

(b) The Index of Dispersion analysis of the 393 spot plate analyses indicated that an average of 8.9% of the samples exceeded the $D^2$ (0.05) value. The American Society for Testing of Materials (ASTM 1976) recommends that unacceptable $D^2$ values not exceed 15% for a method to be considered acceptable.

The percent unacceptable $D^2$ ranged from 4.2% for the Toronto Harbour survey to 14.5% for the Lake Erie survey. Thus all the sets of data used fell within acceptable $D^2$ levels, indicating that the spot plate technique is satisfactory under normal conditions.

One factor which was apparent in studying the $D^2$ values was that the ability and training of the technician is of great importance. This is a subjective observation made during the compiling of the $D^2$ data and is well illustrated by the data set from the "Preliminary Media Comparison Experiment". This experiment involved four technicians each carrying out 24 analyses. Of the 24 results one technician had unacceptable $D^2$ values on 4 analyses (16.7%), two technicians had 3 unacceptable $D^2$ values (12.5%) and one technician had no unacceptable $D^2$ values. The $D^2$ results at least approximated the level of competence previously shown by the technicians involved. Therefore, a technician must become thoroughly familiar with the spot plate technique before using it to perform sample analyses.

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One factor which renders the spot plate technique less accurate is the presence of large spreading colonies such as Proteus sp. and Bacillus sp. This problem was encountered during one survey at the mouth of the Grand River in Lake Erie. During this survey, it was noted that 44 of 104 plates, with the average count of two spots lying in the 5 to 75 range, were overgrown with spreading colonies which have been tentatively identified as Bacillus cereus and an atypical Proteus morganii. The Proteus overgrowth was most common and occurs sporadically in other areas.

The $D^2$ values at an unacceptable level constituted 25 of the 44 plates with large spreading colonies (56.8%), for the remaining 60 plates that were "normal" or had only small spreading colonies, there were 9 (15.0%) unacceptable $D^2$ values.

In situations such as the above, it seems necessary to use an alternative plating technique. It may be possible to perform spot plate analyses in divided or separate petri dishes, or to adopt the spread plate technique. These alternatives will not however overcome the problem caused by spreading colonies but will decrease the degree of cross-contamination between spots.

## 2. Preliminary Media Comparison Experiment

The ANOVA table is included in the Appendix, Table B. The results of the ANOVA indicated that there were no significant differences between HB counts obtained by the four technicians or between duplicates of the same sample on the same media, but there were significant differences between counts obtained for different samples and on different media.

HB recoveries on the eight media are indicated in Table VI.

Table VI: Log GM Recoveries Obtained on Each of Eight Media Tested

| Medium | Actidione | Log GM |
|---|---|---|
| 3.0 g Foot & Taylor | - | 6.050 |
| 3.0 g Foot & Taylor | + | 6.076 |
| 1.0 g Foot & Taylor | - | 6.122 |
| 1.0 g Foot & Taylor | + | 6.117 |
| 0.5 g Foot & Taylor | - | 6.120 |
| 0.5 g Foot &Taylor | + | 6.138 |
| CPS | - | 6.089 |
| CPS | + | 6.091 |

The results of the Tukey's t-test are included in Tables VII and VIII where n = 138, t (0.05) = 3.12 and MS (Error) = 0.0478:

Table VII: Comparison of Media With and Without Actidione (A)

| Medium | 0.5 g - A | 1.0 g - A | 3.0 g - A | CPS - A |
|---|---|---|---|---|
| 0.5 g + A | 3.0 (NSD) | | | |
| 1.0 g + A | | 0.65 (NSD) | | |
| 3.0 g + A | | | 2.12 (NSD) | |
| CPS + A | | | | 0.32 (NSD) |

Table VIII: Comparison of Media With Actidione

| Medium | 3.0 g + A | 1.0 g + A | 0.5 g + A |
|---|---|---|---|
| 1.0 g + A | 4.92 (SD) | | |
| 0.5 g + A | 7.43 (SD) | 2.51 (NSD) | |
| CPS + A | 1.86 (NSD) | 3.05 (NSD) | 5.56 (SD) |

The results of this experiment indicated that the use of Actidione in the media tested did not significantly alter the counts of heterotrophic bcteria. The media (+ Actidione) ranked from highest to lowest HB recoveries are:

$$0.5\ g + A \geqslant 1.0\ g + A \geqslant CPS + A \geqslant 3.0\ g + A.$$

However, if the media are ranked according to the statistical significance of the differences (Table VIII), the following pattern results:

$$0.5\ g + A \quad = \quad 1.0\ g + A$$

$$0.5\ g + A \quad > \quad CPS + A$$

$$1.0\ g + A \quad = \quad CPS + A$$

$$1.0\ g + A \quad > \quad 3.0\ g + A$$

$$CPS + A \quad = \quad 3.0\ g + A$$

In evaluating the study data, it was felt that a final decision could not be made on an optimal media formulation for the following reasons:

(a) No one media was significantly better than all other media.

(b) The data was obtained from only three samples.

(c) Problems were encountered with the counting of colonies on the 3.0 g and CPS media because of large colony size, particularly on the CPS medium.

(d) If the results were separated and analyzed statistically according to sample source, there was a different pattern of recovery for each one:

Lake Ontario

(i) Order of recovery from Highest to Lowest

CPS $\geqslant$ 0.5 g $\geqslant$ 1.0 g > 3.0 g

(ii) Statistical significant of Results (0.05 significance level)

CPS = 0.5 g = 1.0 g > 3.0 g

Humber River

(i) Order of Recovery

1.0 g $\geqslant$ 0.5 g $\geqslant$ CPS g $\geqslant$ 3.0 g

(ii) Statistical Significance

1.0 g = 0.5 g, 1.0 g > CPS

0.5 g = CPS, 0.5 g > 3.0 g

CPS = 3.0 g

Rouge River

(i) Order of Recovery

0.5 g $\geqslant$ 1.0 g > 3.0 g $\geqslant$ CPS g

(ii) Statistical Significance

0.5 g = 1.0 g > 3.0 g = CPS g

3. Second Media Comparison Experiment

The ANOVA table is included in the Appendix, Table C. The results of the ANOVA indicated that there were significant differences between samples and between media. HB recoveries obtained on the four media compared are indicated in Table IX.

Table IX: Log GM Heterotrophic Bacterial Recoveries for Each of Four Media Tested

| Medium | Log GM |
|---|---|
| CPS | 4.745 |
| 3.0 g | 4.706 |
| 1.0 g | 4.700 |
| 0.5 g | 4.518 |

The results of the Tukey's t-test analyses are included in Table X where n = 324, t (0.05) = 2.58 and MS (Error) = 0.0535.

Table X: Comparison of Heterotrophic Bacterial Recoveries on Four Experimental Media

| Media | 0.5 g | 1.0 g | 3.0 g |
|---|---|---|---|
| 1.0 g | 9.95 (SD) | | |
| 3.0 g | 10.33 (SD) | 0.38 (NSD) | |
| CPS | 12.46 (SD) | 2.51 (NSD) | 213 (NSD) |

The media in order from highest to lowest recoveries of HB were:

CPS $\gg$ 3.0g $\gg$ 1.0 g $>$ 0.5 g.

The results of the Tukey's t-test (Table X), however, indicated that only the 0.5 g medium had HB recoveries significantly lower than any of the other media tested.

It is of interest to note that the performance of the media based on HB recoveries for the preliminary and second media experiments were almost in reverse order. A major contributing factor could very well be the difference in experimental design between the two studies including different sources of the samples. As indicated earlier, the preliminary study was based on data obtained from only three samples whereas the second experiment involved the use of 109 samples, thus providing a larger data base.

A further modification made in designing the second media study was to increase the agar concentration in the CPS medium because of a serious problem with large spreading colonies. The modification appeared to be successful as the problem did not reoccur in the second study. This could explain the major improvement in the performance of the CPS medium.

Colony size on the 3.0 g medium was still larger than on the other media, however, the problem did not seem as accute as in the preliminary study and this may have contributed to the improved performance. The reduced proportion of large spreading colonies may be related to the better water quality of the samples used for the second media study (Tables VI and IX). The change in experimental design would also be a factor in the difference in results between the two studies, particularly as no modifications were made to the formulation of the 3.0 g medium.

In the preliminary study the 0.5 g medium had better HB recoveries than the 1.0 g medium over the three samples, however, this difference was not significant and in fact the 1.0 g medium had higher recoveries for one of the samples. In the second study, the HB recoveries on the 1.0 g medium were significantly higher than that for the 0.5 g medium. Since no alterations were made to either medium, the change in performance was probably due to the alterations in experimental design and sampling locations.

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Colony morphology and pigment also varied depending on the media used. The colonies on the 0.5 g and 1.0 g media generally were small and white to light grey with a few yellow colonies. Colonies on the 3.0 g media were for the most part large and yellow. Those on the CPS media were of a variety of sizes and colour (white, grey, yellow, pink, orange, dark-blue, etc.). It is not known whether the increase in colony variability on CPS medium is due to an increase in the number of different species of bacteria isolated or due to the effect of media composition with no change in species variability. The latter is more likely to be the most important factor as a major increase in the types of bacteria isolated could cause a significant increase in the count obtained on CPS and this only held true in the comparison with the 0.5 g medium. In any case, this quality of the CPS medium would be of benefit in any study on the distribution and densities of different bacterial genera and species in water.

In order to further evaluate the performance of the four media, the data were analyzed to determine if the relative recovery of the media varied with the log GM of the samples or with the body of water from which the samples were taken. This analysis was carried out using the FRS test.

The log GM for each sample and media combination was divided in half-log frequency ranges (Table XI).

Table XI: Distribution of Samples from Second Media Comparison Experiment According to Log GM

| | Log GM Range | No. of Samples |
|---|---|---|
| 1 | 3.00 - 3.49 | 3 |
| 2 | 3.50 - 3.99 | 16 |
| 3 | 4.00 - 4.49 | 19 |
| 4 | 4.50 - 4.99 | 24 |
| 5 | 5.00 - 5.49 | 19 |
| 6 | 5.50 - 5.99 | 10 |
| 7 | 6.00 - 6.49 | 2 |

Due to the lack of data in range 1 and 7, these were combined with ranges 2 and 6 respectively.

The results of the FRS analysis are included in Tables XII and XIII. The actual ranks for each sample in each log range, the difference between sample ranks and levels of significance are in the Appendix, Table D to H.

Table XII: Order of Ranks from Lowest to Highest for Each Medium over Log GM Ranges from 3.00 to 6.49

| Log GM Range | Rank Order |
|---|---|
| 3.00 - 3.99 | $R_{CPS} \leq R_{0.5} \leq R_{1.0} \leq R_{3.0}$ |
| 4.00 - 4.49 | $R_{CPS} \leq R_{1.0} \leq R_{3.0} \leq R_{0.5}$ |
| 4.50 - 4.99 | $R_{3.0} \leq R_{CPS} \leq R_{1.0} \leq R_{0.5}$ |
| 5.00 - 5.49 | $R_{CPS} \leq R_{1.0} \leq R_{3.0} \leq R_{0.5}$ |
| 5.50 - 6.49 | $R_{3.0} \leq R_{CPS} \leq R_{1.0} \leq R_{0.5}$ |

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Table XIII: Results of Rank Sums Analysis for Second Media Comparison Study over Log GM Ranges from 3.00 - 6.49

| Rank Differences | LOG GM RANGE 3.00-3.99 | 4.00-4.49 | 4.50-4.99 | 5.00-5.49 | 5.50-6.49 |
|---|---|---|---|---|---|
| $\|R_{CPS} - R_{3.0}\|$ | $SD_{.10}$ | $SD_{.10}$ | NSD | NSD | NSD |
| $\|R_{CPS} - R_{1.0}\|$ | NSD | NSD | NSD | NSD | NSD |
| $\|R_{CPS} - R_{0.5}\|$ | NSD | $SD_{.05}$ | NSD | $SD_{.05}$ | NSD |
| $\|R_{3.0} - R_{1.0}\|$ | NSD | NSD | NSD | NSD | NSD |
| $\|R_{3.0} - R_{0.5}\|$ | NSD | NSD | $SD_{.10}$ | NSD | BSD |
| $\|R_{1.0} - R_{0.5}\|$ | NSD | NSD | NSD | NSD | NSD |

NSD = Non Significant Difference

$SD_{.10}$ = Significant Difference at Level Indicated

The results of Table XII and XIII do not demonstrate any apparent trends related to change in log GM nor do they demonstrate any medium with consistenly higher recoveries than the other media tested. Due to the small differences noted between the four media, the data from Tables XII and XIII were summarized in Table XIV to assist in their further evaluation.

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Table XIV: Overall Performance of Media over Log GM Ranges 3.00 to 6.49

| Media | Number of Times Ranked as | | | | Number of HB Recoveries Higher than | | | | Number of HB Recoveries Significantly Higher than | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | CPS | 3.0 | 1.0 | 0.5 | CPS | 3.0 | 1.0 | 0.5 |
| CPS | 3 | 2 | 0 | 0 | - | 3 | 5 | 5 | - | 2 | 0 | 2 |
| 3.0 | 2 | 1 | 2 | 1 | 2 | - | 2 | 4 | 0 | - | 0 | 1 |
| 1.0 | 0 | 2 | 3 | 0 | 0 | 3 | - | 4 | 0 | 0 | - | 0 |
| 0.5 | 0 | 1 | 0 | 4 | 0 | 1 | 1 | - | 0 | 0 | 0 | - |

The results (Tables XII & XIII) indicate that at any one given log GM range there is more than one medium that would give acceptable results and there are few significant differences between the counts obtained on the four media. However, if the performance of the media shown in Table XIV are considered, it would appear that, for general purpose use, CPS would be the medium of choice as it always had the highest or second highest recoveries. For individual bodies of water however, other media may perform as well and for certain special studies such as in very clean waters (log GM $10^3$/ ml) or those with very high bacterial counts (log GM $10^7$/ ml), other media, such as the 0.5 g and the 3.0 g media, respectively may be better. Unfortunately the samples collected did not extend into these log GM ranges so this possibility could not be evaluated.

The performance of the media was also analyzed by the FRS method to determine if relative recoveries were dependent on the area being studied. The data was utilized for ranking and statistical analysis in three ways:

(a) For each medium the log GM HB recovery was determined for each survey area and these log GM recoveries were then used to determine the rankings for the media over the eight areas.

(b) The media were ranked for each survey area separately by using the HB counts obtained on each medium for each sample of the survey area being examined.

(c) The media were ranked over all survey areas by using the HB counts obtained on each medium for all 109 samples analyzed.

The results are summarized in Tables XV and XVI. Detailed results are included in the Appendix, Table I to R.

Table XV: Order of Ranks from Lowest-to Highest for each Medium as Determined for Different Survey Areas

| Area | | Rank Order |
|---|---|---|
| i) All Survey Areas | | $R_{CPS} \leq R_{1.0} \leq R_{3.0} \quad R_{0.5}$ |
| ii) Individual Areas | | |
| a) Burlington | (Log GM = 4.287) | $R_{3.0} \leq R_{1.0} \leq R_{CPS} = R_{0.5}$ |
| b) Peel | (Log GM = 4.997) | $R_{0.5} \leq R_{CPS} \leq R_{1.0} \leq R_{3.0}$ |
| c) Etobicoke | (Log GM = 4.962) | $R_{CPS} \leq R_{1.0} < R_{3.0} < R_{0.5}$ |
| d) Scarborough | (Log GM = 4.772) | $R_{CPS} \leq R_{1.0} \leq R_{3.0} \leq R_{0.5}$ |
| e) Toronto | (Log GM = 5.044) | $R_{CPS} = R_{3.0} < R_{1.0} \leq R_{0.5}$ |
| f) Twelve Mile Creek | (Log GM = 4.930 | $R_{CPS} \leq R_{1.0} < R_{0.5} \leq R_{3.0}$ |
| g) Hamilton Bay | (Log GM = 4.510) | $R_{3.0} = R_{CPS} \leq R_{1.0} \leq R_{0.5}$ |
| h) Lake Ontario | (Log GM = 3.602) | $R_{CPS} \leq R_{0.5} \leq R_{3.0} \leq R_{1.0}$ |
| iii) All samples | | $R_{CPS} \leq R_{3.0} \leq R_{1.0} \leq R_{0.5}$ |

Table XVI: Results of Rank Sums Analysis for the Second Media Comparison Study over Different Survey Areas

| Rank Differences | SURVEY AREA: All Areas | Burlington | Peel | Etobicoke | Scarborough | Toronto | Twelve Mile Creek | Hamilton Bay | Lake Ontario | All Samples |
|---|---|---|---|---|---|---|---|---|---|---|
| $\lvert R_{CPS} - R_{3.0} \rvert$ | NSD | NSD | NSD | NSD | NSD | NSD | $SD_{.05}$ | NSD | NSD | $SD_{.05}$ |
| $\lvert R_{CPS} - R_{1.0} \rvert$ | NSD | NSD | NSD | NSD | NSD | $SD_{.05}$ | NSD | NSD | $SD_{.05}$ | $SD_{.05}$ |
| $\lvert R_{CPS} - R_{0.5} \rvert$ | $SD_{.05}$ | NSD | NSD | $SD_{.05}$ | $SD_{.05}$ | $SD_{.05}$ | NSD | $SD_{.05}$ | NSD | $SD_{.05}$ |
| $\lvert R_{3.0} - R_{1.0} \rvert$ | NSD | NSD | NSD | NSD | NSD | $SD_{.05}$ | $SD_{.10}$ | NSD | NSD | NSD |
| $\lvert R_{3.0} - R_{0.5} \rvert$ | NSD | NSD | $SD_{.05}$ | $SD_{.05}$ | NSD | $SD_{.05}$ | NSD | $SD_{.05}$ | NSD | NSD |
| $\lvert R_{1.0} - R_{0.5} \rvert$ | NSD | NSD | NSD | $SD_{.05}$ | $SD_{.10}$ | NSD | NSD | NSD | NSD | NSD |

NSD = Non Significant Difference; $SD_{.10}$ = Significant Difference at Level Indicated

The performance of the media based on the log GM of each survey area (Table XV, i) was comparable to the results obtained by the ANOVA-student t-test analysis (Tables IX & X). The only difference between the two was a reversal in order of the relative performance of the 3.0 g and 1.0 g media. In neither case was the difference in performance of the two media significant.

When the samples in each individual survey area were analyzed statistically (Table XV, ii), the relative performance of the media was variable with no consistent order of recovery being noticeable from area to area. There were few significant differences between recoveries on the different media over the eight survey areas, so this data was further summarized in Table XVII to assist in its evaluation.

Table XVII: Overall Performance of Media for Samples in each of Eight Survey Areas

| Media | Number of Times Ranked as | | | | Number of Times HB Recoveries Higher than | | | | Significantly Higher than | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | CPS | 3.0 | 1.0 | 0.5 | CPS | 3.0 | 1.0 | 0.5 |
| CPS | 6 (2=) | 1 | 1 (1=) | 0 | - | 5 | 7 | 6 | - | 1 | 2 | 4 |
| 3.0 | 3 (2=) | 0 | 4 | 1 | 1 | - | 4 | 6 | 0 | - | 1 | 3 |
| 1.0 | 0 | 4 | 3 | 1 | 1 | 4 | - | 6 | 0 | 1 | - | 2 |
| 0.5 | 1 | 1 | 1 (1=) | 5 | 1 | 2 | 2 | - | 0 | 1 | 0 | - |

(2 =, 1 =) : indicates equal ranking.

Again the results of the FRS analysis (Tables XV, ii & XVI) indicate that for each individual survey area there was more than one medium that would give satisfactory results (Ranks NSD). However, when the results for all eight survey areas (Table XVII) are considered, the medium which provides the most consistently acceptable results is CPS. The reasons for other media performing

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better in certain areas is not apparent from this data and it does not appear to reflect the GM of the different bodies of water. The 3.0 g medium ranked well in three survey areas for which the log GM's were 4.287, 4.511 and 5.044. The only case in which the 0.5 g media proved satisfactory was in an area with a log GM of 4.997. The performance of a given medium is probably dependent on the number of factors including general bacterial water quality, source of bacteria and physical and chemical characteristics of the water body being studied. The relative performance of the media (Table XV, iii) as determined by the FRS analysis when ranking was performed over all 109 samples (Table XV, iii) is identical to that obtained by the ANOVA-Student t-test analysis (Tables IX & X). The CPS medium had the lowest rank of the four media (Table XVI) and the rank differences between CPS and each of the other media were significantly different from zero (Appendix, Table R). There was no significant difference in the performance of the 3.0 g and 1.0 g media. Therefore, these final results provide further evidence that CPS would be the best choice as a general purpose heterotrophic bacterial medium for surface water analysis.

## Conclusion

The data comparing plating techniques demonstrated that the spot plate technique compares favourably with the spread plate technique and that both were superior to the membrane filtration and pour plate techniques. Thus the spot plate technique can be used for water quality testing providing two conditions are met:

1) Bacterial species forming large spreading colonies must not constitute a significant part of the overall bacterial population, In such a case, alternative approaches must be considered that either contain or inhibit the spreading.

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2) Before personnel employ this technique on a routine basis, thorough training must be provided.

The experiment on the incorporation of Actidione into the heterotrophic bacterial media demonstrated that the concentration of 100 ppm could be used without lowering bacterial recoveries.

Of the four media tested, the CPS formulation gave the most satisfactory performance overall. The experiments indicated that for certain studies, media formulations other than CPS may provide higher recoveries, however, for routine studies the CPS medium is recommended.

Therefore, the spot plate technique in conjunction with CPS Agar + 100 ppm Actidione may be used successfully for the routine heterotrophic bacterial analysis of surface water samples.

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## Media Formulation

Foot and Taylor Agar

| Ingredients | Amount/litre |
|---|---|
| $K_2HPO_4$ | 0.2 g |
| $MgSO_4$ | 0.05 g |
| Ferric Chloride | 0.2 ml |
| Peptone | 0.5 g |
| Soluble Casein | 0.5 g |
| Agar | 20.0 g |
| Distilled Water | 1000 ml |

final pH 7.2

(i) Foot and Taylor Agar — 0.5 g peptone

Formulation as above

(ii) Foot and Taylor Agar — 0.5 g peptone + Actidione

Same as (i) with the addition of 100 ppm Actidione

(iii) Foot and Taylor Agar — 1.0 g peptone

Same as (i) except that 1.0 g peptone is added

(iv) Foot and Taylor Agar — 1.0 g peptone + Actidione

Same as (iv) with the addition of 100 ppm Actidione

(v) Foot and Taylor Agar — 3.0 g peptone

Same as (i) except that 3.0 g peptone is added

(vi) Foot and Taylor Agar — 3.0 g peptone + Actidione

Same as (v) with the addition of 100 ppm Actidione

(vii) CPS Agar

Same as (i) with the addition of 0.5 g Soluble Starch and 1.0 g of Glycerol

(viii) CPS Agar + Actidione

Same as (vii) with the addition of 100 ppm Actidione

TABLE A: ANOVA RESULTS for COMPARISON of

PLATING TECHNIQUES EXPERIMENT

| SOURCE | df | SS | MS | F | F (0.05) |
|---|---|---|---|---|---|
| METHOD (M) | 3 | 20.0110 | 6.6703 | 49.82* | 2.60 |
| SAMPLES (S) | 83 | 176.2032 | 2.1229 | 15.85* | 1.22 |
| ERROR (MxS) | 249 | 33.3431 | 0.1339 | | |

* = Significant at 0.05 Confidence Level

TABLE B: ANOVA RESULTS for PRELIMINARY MEDIA COMPARISON EXPERIMENT

| SOURCE | df | SS | MS | F | F (0.05) |
|---|---|---|---|---|---|
| MEDIA (M) | 7 | 0.1408 | 0.0201 | 2.45* | 2.24 |
| SAMPLE (S) | 2 | 49.7090 | 24.8545 | 3031.04* | 5.15 |
| M x S | 14 | 0.1287 | 0.0092 | 1.12 | 1.94 |
| TECHNICIAN (T) | 3 | 0.0191 | 0.0064 | 0.78 | 2.83 |
| T x M | 21 | 0.1322 | 0.0063 | 0.77 | 1.78 |
| T x S | 6 | 0.1065 | 0.0178 | 2.17 | 2.32 |
| T x M x S | 42 | 0.3460 | 0.0082 | 2.48* | 1.69 |
| DUPLICATE (D) | 1 | 0.0002 | 0.0002 | 0.06 | 4.08 |
| D x S | 7 | 0.0273 | 0.0039 | 1.18 | 2.25 |
| S x M | 2 | 0.0191 | 0.0096 | 2.91 | 3.23 |
| D x S x M | 14 | 0.0193 | 0.0014 | 0.42 | 1.92 |
| D x T | 3 | 0.0068 | 0.0023 | 0.70 | 2.84 |
| D x S x T | 21 | 0.0741 | 0.0035 | 1.06 | 1.84 |
| D x M x T | 6 | 0.0285 | 0.0047 | 1.42 | 2.34 |
| ERROR (D x S x M x T) | 42 | 0.1389 | 0.0033 | | |

Note: As the interaction term T x M x S was significant at the 0.05 confidence level, this term was used to determine F values for all terms containing T or M or S.

* = Significant at 0.05 Confidence Level.

TABLE C: ANOVA RESULTS for SECOND MEDIA COMPARISON EXPERIMENT

| SOURCE | df | SS | SM | F | F (0.05) |
|---|---|---|---|---|---|
| MEDIA (M) | 3 | 3.2092 | 1.0697 | 19.10* | 2.60 |
| SAMPLE (S) | 108 | 211.7183 | 1.9604 | 36.64* | 1.22 |
| ERROR (M x S) | 324 | 17.3272 | 0.0535 | | |

* = Significant at 0.05 Confidence Level

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**TABLE D: Ranks and Rank Differences for the Second Media Comparison Experiment**

**Over Log GM Range 3.00 - 3.99**

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 38 | | | |
| 3.0 g | 36 | 18** | | |
| 1.0 g | 54 | 16*** | 2 | |
| 0.5 g | 42 | 4 | 14 | 12 |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 20$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 18$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 16$

TABLE 8: Ranks and Rank Differences for the Second Media Comparison Experiment Over Log GM Range 4.00 - 4.49

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 34 | | | |
| 3.0 g | 53 | 19** | | |
| 1.0 g | 46 | 12 | 7 | |
| 0.5 g | 55 | 21* | 2 | 9 |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 20$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 18$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 16$

TABLE F: Ranks and Rank Differences for the Second Media Comparison Experiment Over Log GM Range 4.50 - 4.99

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) SPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 53 | | | |
| 3.0 g | 51 | 2 | | |
| 1.0 g | 64 | 11 | 13 | |
| 0.5 g | 72 | 19*** | 21** | 8 |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 23$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 20$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 18$

TABLE G : Ranks and Rank Differences for the Second Media Comparison Experiment

Over Log GM Range 5.00 - 5.99

| MEDIA | RANK | Difference in Ranks ($\mid R_1 - R_2 \mid$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 32 | - - - - - | | |
| 3.0 g | 48 | 16*** | | |
| 1.0 g | 47 | 15*** | 1 | |
| 0.5 g | 63 | 31* | 15*** | 16*** |

$* \mid R_1 - R_2 \mid_{(0.05)} = 20$

$** \mid R_1 - R_2 \mid_{(0.10)} = 18$

$*** \mid R_1 - R_2 \mid_{(0.20)} = 15$

TABLE H: Ranks and Rank Differences for the Second Media Comparison Experiment

Over Log GM Range 5.50 - 6.49

| MEDIA | RANK | Difference in Ranks ($I R_1 - R_2 I$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 28 | | | |
| 3.0 g | 26 | 2 | | |
| 1.0 g | 29 | 1 | 3 | |
| 0.5 g | 37 | 9 | 11 | 8 |

* $I R_1 - R_2 I_{(0.05)} = 16$

** $I R_1 - R_2 I_{(0.10)} = 14$

*** $I R_1 - R_2 I_{(0.20)} = 12$

TABLE I: Ranks and Rank Differences for the Second Media Comparison Experiment Over All Eight Survey Areas

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 14 | | | |
| 3.0 g | 20 | 6 | | |
| 1.0 g | 19 | 5 | 1 | |
| 0.5 g | 27 | 13* | 7 | 8 |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 13$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 12$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 10$

TABLE J: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Burlington Survey Area

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 48 | | | |
| 3.0 g | 35 | 13 | | |
| 1.0 g | 39 | 9 | 4 | |
| 0.5 g | 48 | 0 | 13 | 9 |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 19$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 17$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 15$

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TABLE K: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Peel Survey Area

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 15 | — | | |
| 3.0 g | 22 | 7 | | |
| 1.0 g | 16 | 1 | 6 | |
| 0.5 g | 7 | 8 | 15* | 9*** |

$* \ \lvert R_1 - R_2 \rvert_{(0.05)} = 11$

$** \ \lvert R_1 - R_2 \rvert_{(0.10)} = 10$

$*** \ \lvert R_1 - R_2 \rvert_{(0.20)} = 9$

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TABLE L: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Etobicoke Survey Area

| MEDIA | RANK | Difference in Ranks ($\mid R_1 - R_2 \mid$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 34.5 | | | |
| 3.0 g | 41.5 | 7 | | |
| 1.0 g | 40.5 | 6 | 1 | |
| 0.5 g | 63.5 | 29* | 22* | 23* |

$* \mid R_1 - R_2 \mid_{(0.05)} = 20$

$** \mid R_1 - R_2 \mid_{(0.10)} = 18$

$*** \mid R_1 - R_2 \mid_{(0.20)} = 15$

TABLE M: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Scarborough Survey Area

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 22 | | | |
| 3.0 g | 32 | 10 | | |
| 1.0 g | 26 | 4 | 6 | |
| 0.5 g | 40 | 18* | 8 | 14** |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 16$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 14$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 12$

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TABLE N: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Toronto Survey Area

| MEDIA | RANK | Difference in Ranks ($\mid R_1 - R_2 \mid$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 38.25 | | | |
| 3.0 g | 38.25 | 0 | | |
| 1.0 g | 60.25 | 22* | 22* | |
| 0.5 g | 63.25 | 25* | 25* | 3 |

$* \mid R_1 - R_2 \mid_{(0.05)} = 2$

$** \mid R_1 - R_2 \mid_{(0.10)} = 19$

$*** \mid R_1 - R_2 \mid_{(0.20)} = 16$

TABLE α: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Twelve Mile Creek Survey Area

| MEDIA | RANK | Difference in Ranks ($\mid R_1 - R_2 \mid$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 28 | | | |
| 3.0 g | 55 | 27* | | |
| 1.0 g | 37 | 9 | 18** | |
| 0.5 g | 41 | 13 | 14*** | 4 |

$* \mid R_1 - R_2 \mid_{(0.05)} = 19$

$** \mid R_1 - R_2 \mid_{(0.10)} = 17$

$*** \mid R_1 - R_2 \mid_{(0.20)} = 14$

TABLE P: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Hamilton Bay Survey Area

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 15 | - - - - | | |
| 3.0 g | 15 | 0 | | |
| 1.0 g | 21 | 6 | 6 | |
| 0.5 g | 28 | 13* | 13* | 7** |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 13$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 12$

*** $\lvert R_1 \; R_2 \rvert_{(0.20)} = 10$

TABLE Q: Ranks and Rank Differences for the Second Media Comparison Experiment for Samples from Lake Ontario Survey Area

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) | | |
|---|---|---|---|---|
| | | CPS | 3.0 | 1.0 |
| CPS | 18 | | | |
| 3.0 g | 30 | 12*** | | |
| 1.0 g | 38 | 20* | 8 | |
| 0.5 g | 25 | 7 | 5 | 13*** |

$* \lvert R_1 - R_2 \rvert_{(0.05)} = 16$

$** \lvert R_1 - R_2 \rvert_{(0.10)} = 14$

$*** \lvert R_1 - R_2 \rvert_{(0.20)} = 12$

**TABLE R: Ranks and Rank Differences for the Second Media Comparison Experience Over All 109 Samples**

| MEDIA | RANK | Difference in Ranks ($\lvert R_1 - R_2 \rvert$) CPS | 3.0 | 1.0 |
|---|---|---|---|---|
| CPS | 219 | | | |
| 3.0 g | 278 | 59* | | |
| 1.0 g | 279 | 60* | 1 | |
| 0.5 g | 316 | 97* | 38*** | 37*** |

* $\lvert R_1 - R_2 \rvert_{(0.05)} = 49$

** $\lvert R_1 - R_2 \rvert_{(0.10)} = 44$

*** $\lvert R_1 - R_2 \rvert_{(0.20)} = 37$